Insulin affects only initiation and not elongation in protein synthesis in soleus muscles of lean and obese mice

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The effect of insulin on polypeptide chain initiation and elongation has been studied in soleus muscles isolated from lean and goldthioglucose-obese mice. Insulin increased the amount of radioactivity present in nascent chains by ~30% in muscles from both lean and obese mice, indicating that it stimulates peptide chain initiation. In contrast, elongation rates, estimated by measurement of half transit time, were similar in basal conditions and insulin-treated muscles of lean and obese animals. Thus, insulin increased the initiation without modifying the elongation rates. Obesity did not affect either basal rates of initiation and elongation or the effect of insulin.

Insulin *Protein synthesis* *Skeletal muscle* *Initiation* *Elongation* *Obesity*

1. INTRODUCTION

A role for insulin in stimulating protein synthesis has been known for a long time. Protein synthesis results from a array of reactions: in the translation process, which follows transcription, one distinguishes three major steps; peptide chain initiation, elongation and termination [1]. It is generally assumed that insulin is involved in the regulation of polypeptide chain initiation and does not affect elongation. This view essentially stems from analysis of ribosomal profiles [2,3]. Here, we have looked more directly for an insulin effect on initiation and elongation steps in skeletal muscle incubated in vitro:

(1) By using pactamycin which specifically blocks initiation of new peptide chains without preventing elongation of the chains already initiated before addition of the drug [4,5];
(2) By measuring the transit time of the average mRNA, i.e., the time that elapsed between the initiation of protein synthesis and the release of completed polypeptides.

Experiments have been performed in normal mice and in mice rendered obese, hyperinsulinemic and insulino-resistant by goldthioglucose injection [6]. We show here that in soleus muscles:

(1) Insulin increased initiation rate without modifying the average rate of polypeptide elongation in normal lean mice;
(2) Obesity did not impair basal rates of initiation or elongation and the effects of insulin.

Abbreviation: GTG, goldthioglucose

2. MATERIALS AND METHODS

2.1. *Isolation of soleus muscle and incubation procedure*

Soleus muscles, isolated from 20-week-old lean and goldthioglucose obese mice [7], were first incubated for 120 min in 1 ml Krebs–Ringer bicarbonate buffer containing 2 mg/ml defatted bovine serum albumin, 2 mM pyruvate and [^{3}H]leucine (2 μCi/ml, 60 Ci/mmol). [^{14}C]Leucine (1 μCi/ml, 300 mCi/mmol) was then added and incubation continued for 1–15 min. When required, insulin (6.7 nM), was present during the last 20 min of the first incubation and throughout the second incubation.

2.2. *Determination of nascent polypeptide chains*

Pactamycin (2 μM) (a gift from the Upjohn

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Company, Kalamazoo MI) was added at the onset of the second incubation, together with [^{14}C]-leucine. At the end of incubation, muscles were freeze-clamped and sonicated in ice-cold potassium phosphate buffer (10 mM, pH 7.35) containing 1 mM phenylmethylsulfonyl-fluoride (PMSF). Homogenates were centrifuged for 10 min at 8000 × *g*. Labeled proteins present in the soluble fraction (released + nascent polypeptides) were determined from the amount of ^{14}C/^{3}H radioactivity present in the material precipitable by hot (10%) trichloracetic acid [4,5].

2.3. *Determination of transit times*

After incubation and homogeneisation of muscles as above, transit times were determined as in [5]. Briefly ^{14}C/^{3}H ratios in trichloracetic acid precipitable proteins were determined in 8000 × *g* supernatants (nascent + released polypeptides) and in 150 000 × *g* supernatants (released polypeptides). Transit time calculations were done by extrapolating to zero the plot of the ratio of ^{14}C/^{3}H (using a least-squares analysis) *vs* time for the two fractions; the time obtained for the released proteins was then subtracted from that obtained for total proteins. Since the average nascent peptide chain is theoretically half-complete at the time of addition of the first [^{14}C]leucine molecule, this observed time difference corresponds to half the actual transit time [8,9].

To overcome variations due to differences between animals, paired soleus muscles were used. Two muscles were isolated from the same mouse, one muscle was incubated in the presence of insulin and the other one was used as a control (basal condition). Further, pre-labelling the proteins with [^{3}H]leucine was carried out to take into account slight variations in isotope uptake and in basal protein synthesis [8].

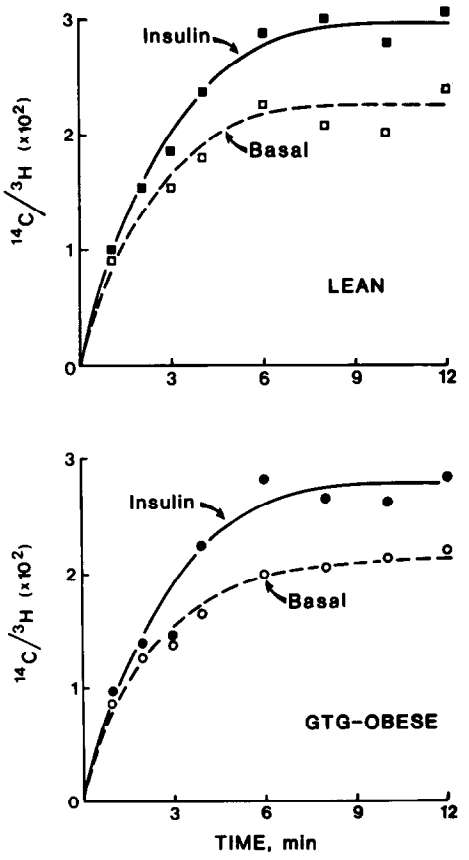

Fig.1. Effect of insulin on the number of nascent chains for total proteins in muscles of lean and GTG-obese mice. Muscles were first incubated for 120 min in Krebs–Ringer bicarbonate buffer containing [^{3}H]-leucine. They were then incubated for the times indicated in the same buffer containing [^{14}C]leucine and 2 μM pactamycin. Insulin (6.7 nM) was present during the last 20 min of the first incubation and during the second incubation. ^{14}C/^{3}H ratios in trichloroacetic acid precipitable proteins of 8000 × *g* supernatants were determined as in section 2.

3. RESULTS

3.1. *Insulin effect on polypeptide chain initiation*

Insulin significantly increased polypeptide chain initiation (fig.1). Thus, the amount of radioactivity present in nascent polypeptide chains after pactamycin addition was increased by 30% in muscles incubated with insulin, compared to muscles incubated in the absence of hormone. This suggests that more chains were initiated in the presence of insulin than in its absence. No differences could be observed between lean controls (fig.1, upper) and goldthioglucose mice (fig.1, lower) in the number of nascent chains and in the degree of stimulation induced by insulin.

3.2. *Measurement of transit times*

We next determined the ribosomal transit times for proteins (fig.2). In muscles from control lean

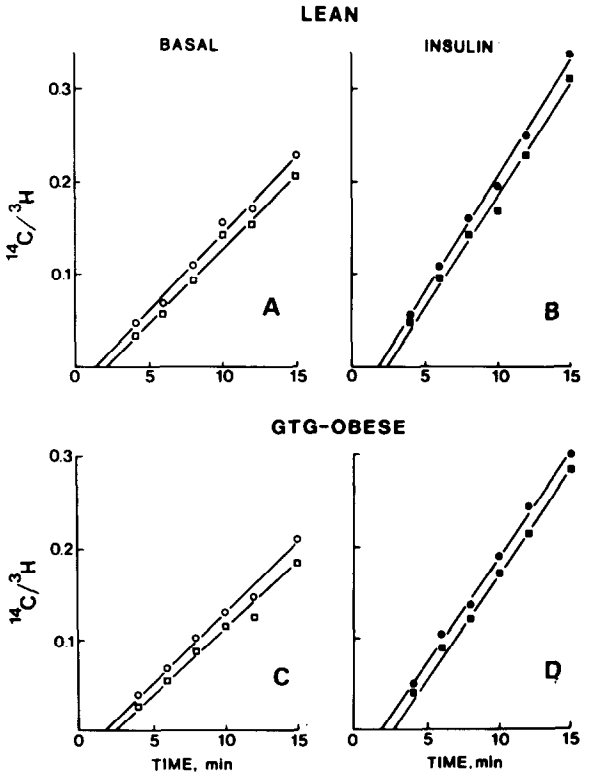

Fig.2. Effect of insulin on ribosomal transit time for total proteins in muscles of lean and GTG-obese mice. Muscles were first incubated as in fig.1, and were then incubated in the same buffer containing [^{14}C]leucine. $^{14}C/^{3}H$ ratios were determined as in section 2 in trichloroacetic acid precipitable proteins of 8000 × *g* supernatants (upper lines), (nascent and released polypeptide chains) and of 150 000 × *g* supernatants (lower lines, released polypeptide chains).

mice, the values of transit times were identical in the absence (A) and presence (B) of insulin: $t_{1/2}$ = 0.5 ± 0.1 min (mean ± SEM of 4 values, obtained in 4 different expt), even though insulin raised protein synthesis by 1.4-times as judged from the slopes of the curves. Transit times were also similar in muscles from GTG-obese mice incubated in basal condition (C, $t_{1/2}$ = 0.6 ± 0.1 min) and in the presence of insulin (D, $t_{1/2}$ = 0.5 ± 0.1 min).

4. DISCUSSION

Here, we have studied insulin effect on the first steps of protein synthesis in muscles, using leucine as a precursor. Insulin stimulated protein synthesis by 30–40%. This stimulatory effect of insulin is analogous to that already reported in the soleus muscle for tyrosine incorporation into muscular proteins [10,12]. It is unlikely that this effect of insulin results from an effect on the specific activity of Leu-tRNA since insulin does not stimulate leucine transport in muscle ([13] and Y.I.M.B., unpublished). Insulin seems to stimulate only the initiation step without modifying the rate of elongation. An alternative mechanism would be an increase in mRNA content in insulin-treated muscle; this is also unlikely since:

(i) The proportion of mRNA in total RNA has been shown to be identical in muscles from controls, diabetics and insulin-treated diabetics [14];

(ii) Insulin is still able to stimulate protein synthesis in diabetic animals pretreated with actinomycin D suggesting that RNA synthesis is not essential for the hormone effect on initiation [2].

Our results agree with those reported in chick embryo fibroblasts where insulin was shown to activate limiting components of the initiation system [15]. Demonstration of an insulin effect on the initiation step in vitro is consistent with the reversal of the blockade of initiation observed in diabetic animals following insulin treatment [2].

We also show here that protein synthesis in muscles isolated from goldthioglucose obese animals and lean controls is similar. This indicates that hyperinsulinemia and insulino-resistance which are characteristic features of these animals [6] do not impair the protein synthesis machinery in soleus muscles. This is in agreement with our observation of a lack of alteration in tyrosine incorporation in total proteins, in basal or insulin-stimulated conditions [11]. Taken together, these results suggest that the decrease in protein content observed in obese animals [16] is not accounted for by a defect in the protein synthesis per se, but rather to some other factors (circulating factors, or exercise) occurring in vivo.

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